Plasma Kinetics and Biodistribution of a Lipid Emulsion Resembling Low-Density Lipoprotein in Patients with Acute Leukemia

Raul C. Maranhão, Bernardo Garicochea, Edson L. Silva, Pedro Dorlihe-Llacer, Silvia M. S. Cadena, Iris J. C. Coelho, José Claudio Meneghetti, Fulvio J. C. Pilleggi, and Dalton A. F. Chamone

The Heart Institute (InCor) and Department of Hematology and Hemotherapy (Hemocentro) of the São Paulo University Medical School Hospital, and Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

ABSTRACT

Low-density lipoprotein (LDL) could be used as a carrier of chemotherapeutic agents to neoplastic cells that overexpress LDL receptors (rLDL), but LDL is difficult to obtain and handle. Recently, it was observed that a protein-free emulsion resembling the lipid portion of LDL (LDE) behave like native LDL when injected into the bloodstream. In this study, the evidence that LDE is taken up by rLDL was expanded by comparing LDL and LDE plasma decay curves in rabbits and by competition experiments with lymphocytes. To verify whether LDE could be removed from the plasma by neoplastic cells with increased rLDL, LDE labeled with $^{3}H$cholesterol ester was injected into 14 patients with acute myeloid leukemia (AML) and into 7 with acute lymphocytic leukemia (ALL). In AML, rLDL expression is increased but in ALL it is normal. LDE plasma fractional clearance rate (FCR, in $h^{-1}$) was calculated from the remaining radioactivity measured in plasma samples collected during 24 h following injection. LDE FCR was 3-fold greater in AML than in ALL patients ($0.192 \pm 0.210$ (SD) and $0.066 \pm 0.033 h^{-1}$, respectively, $P < 0.035$). When LDE injection was repeated in 9 AML patients in hematological remission, LDE FCR diminished 66% compared to the pretreatment values (from $0.192 \pm 0.210$ to $0.065 \pm 0.038 h^{-1}$, $P < 0.02$), so that it could be estimated that nearly 66% of the emulsion was taken up by AML cells and only 34% by the normal tissues. As expected, LDE FCR was unchanged in 4 patients with ALL in hematological remission ($0.069 \pm 0.044 h^{-1}$). Gamma camera images obtained 6 h after the injection of $^{99m}$Tc-labeled LDE into one patient with ALL showed biodistribution similar to that of LDL. In one AML patient LDE was comparatively more concentrated over the areas corresponding to the bone marrow infiltrated by AML cells. Our results indicate that LDE FCR is increased in a disease known to contain malignant cells that overexpress rLDL, suggesting that LDE is taken up by malignant cells with increased rLDL.

INTRODUCTION

LDL carries most of the plasma cholesterol in humans. LDL is a quasiparticle composed of a core of cholesteryl esters surrounded by a monolayer of phospholipids and free cholesterol. Apolipoprotein B-100, the protein part of LDL, is the ligand for binding of the lipoprotein to specific receptors (B, E receptors, rLDL) on the cell plasma membrane. After binding to the receptors, LDL is internalized and degraded in lysosomes (1) and its cholesterol is used in several cell processes, like membrane synthesis.

In the rapidly proliferating malignant cells, the increased need of cholesterol for new membrane synthesis may result in overexpression of the rLDL (2), which allows greater uptake of the lipoprotein by the neoplastic cells. Several investigators showed the uptake of LDL-drug complexes by malignant cells (3-7), leading to the possibility of using LDL as drug carrier in cancer chemotherapy (3, 8-10). This would increase the antineoplastic effects while reducing the toxic effects upon normal cells. However, isolation from plasma and handling of native LDL are difficult; therefore its use in cancer treatment may remain confined to experimental trials (11).

Recently, Maranhão et al. demonstrated that LDE, a protein-free emulsion with composition resembling the lipid portion of LDL, behaves similarly to LDL when injected into rats (12) and human subjects (13). In the present study, the evidence that LDE is taken up by rLDL was further strengthened in studies with rabbits and human leukocytes. LDE labeled with $^{3}H$cholesterol ester was then injected i.v. in two groups of patients: group 1, with AML; and group 2, with ALL. AML cells overexpress rLDL, whereas rLDL activity in ALL is similar to normal cells (2, 14) and the plasma decay curves of the LDE label were determined. The effects of chemotherapy on the LDE clearance were also examined. The images of the biodistribution of LDE labeled with $^{99m}$Tc were obtained in one ALL and in one AML patient. Taken together, our results indicate that LDE removal from plasma is increased in a disease known to contain malignant cells that overexpress rLDL, suggesting that LDE is taken up by malignant cells with increased rLDL.

MATERIALS AND METHODS

Study Subjects. Fourteen patients with AML and 7 with ALL were studied at the Infirmary of the Department of Hematology and Hemotherapy of the São Paulo University Medical School Hospital. The experimental protocol was approved by the Ethics Committee of the São Paulo University Medical School and an informed consent was obtained from each participant. Individual characteristics and clinical data are shown in Table 1. Classification of the acute leukemias was according to the French-American-British criteria (15). When they were first studied, the patients were newly diagnosed. All patients exhibited bone marrow hypercellularity, and chemotherapy had not yet been initiated. In 9 patients with AML the study was repeated after they were treated with induction chemotherapy and achieved complete hematological remission (less than 5% of blasts in the bone marrow biopsy and normal blood cell count). Treatment was performed with daunorubicin (50 mg/m² during 3 days) and 1-β-D-arabinofuranosylcytosine (100 mg/m² during 7 days). Among the remaining 5 patients, complete hematological remission was not obtained in 3 patients, and 2 died during induction therapy. The study was also repeated after remission in 4 of the patients with ALL submitted to treatment according to the following protocol: vincristine, 1.4 mg/m² i.v., at days 1, 8, 15, 22, 28, and 36 of treatment; daunorubicin, 50 mg/m² i.v., at days 1, 2, 3, and 15; prednisone, 60 mg/m² p.o. during the first 28 days of treatment; l-asparaginase, 6000 units/m² i.v., from the 17th to the 28th day of treatment; methotrexate, 12 mg i.v., on the 8th day of treatment. Among the remaining three ALL patients, complete remission was not obtained in one of them during the induction chemotherapy. Both groups of AML and ALL patients had already stopped treatment for at least 10 days when the LDE clearance study was repeated. One ALL and one AML patient were given $^{99m}$Tc LDE for acquisition of biodistribution images. The two patients were newly diagnosed and had not been treated. In those the LDE clearance study was not performed.

Determination of Cholesterol and Triglycerides. The total plasma cholesterol and triglycerides (TG) of the subjects were determined after a 12-h fast with the aid of enzymatic kits (CHOD-PAP, Merck, and Abbott, respectively). HDL cholesterol was determined with the same method, after precipitation of...
LDL and VLDL with MgCl₂ and phosphotungstic acid. VLDL concentration was calculated assuming the relation

\[ \text{VLDL} = \text{TG}/5 \]

(16). LDL was calculated using the Friedewald equation where LDL = total cholesterol – (VLDL + HDL) (17).

Preparation of LDE. Egg phosphatidylcholine was purchased from Lipid Products (Surrey, United Kingdom); triolein, cholesteryl oleate, andcholesterol were from Nu-Chek Prep (Elysian, NJ), and radioactive lipids were from LKB Products (Surrey, United Kingdom); triolein, cholesteryl oleate, and cholesterol were >98% pure as determined by thin layer chromatography. The LDE used in this study was choleseryl oleate – (VLDL + HDL) (17).

The dried lipids were resuspended in 10 ml of 0.1 M KCl-0.01 M Tris-HCl, pH 7.5. The top 20-30% of the sample was collected by aspiration, after attaining the background density of approximately 1.006 g/ml, was removed by aspiration with a needle. The remaining solution was adjusted to a background density of 1.22 g/ml with KBr and analyzed for lipid composition by standard laboratory methods. The top 10% of the solution, containing particles that float at background density of approximately 1.006 g/ml, was removed by aspiration with a needle. The remaining solution was adjusted to a background density of 1.22 g/ml with KBr and analyzed for lipid composition by standard laboratory methods.

LDL for 4 h at 37°C in 1 ml of RPMI. After incubation, the cells were washed three times with RPMI at 4°C and dissolved in 0.2 M NaOH for 2 h at 37°C. Viable cells (10⁶) were incubated with 10 μg of 14C-LDE-apo and increasing concentrations of FCR during 2 h at 37°C. After incubation, the density was adjusted to 1.063 g/ml with KBr and the mixture was submitted to ultracentrifugation at 195,000 x g for 24 h. The emulsion containing apolipoproteins was recovered at the top of the tube and dialyzed against 3 changes of 2 liters of 0.01 M Tris-HCl buffer, containing 0.15 M NaCl and 1 mM EDTA. The resulting artificial lipoprotein (14C-LDE-apo) was submitted to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (22) to analyze the apolipoprotein content and used in the cell binding studies.

Preparation of HDL and LDL. HDL and LDL were obtained from the blood of healthy normolipidemic donors by sequential ultracentrifugation (21). Lipoproteins were isolated from the blood of healthy normolipidemic subjects by centrifugation on a Ficoll-Hypaque gradient. The cells were incubated during 24 h in RPMI 1640 containing 100 units/ml of penicillin, 5 μg/ml of gentamicin, and supplemented with 10% of lipoprotein-deficientserum. This procedure induces the maximal expression of rLDL (2). After this period, the cells were harvested and the viability was evaluated by trypan blue exclusion. Viable cells (10⁶) were incubated with 10 μg of 14C-LDE-apo and increasing concentrations of LDL for 4 h at 37°C in 1 ml of RPMI. After incubation, the cells were washed three times with RPMI at 4°C and dissolved in 0.2 M NaOH for 2 h at 37°C. An aliquot was removed for protein determination (23) and the incorporated radioactivity was determined in the liquid scintillation counter.

Preparation of 241Am-LDE and 32p-Tc-LDL. Mononuclear leukocytes were isolated from the blood of healthy normolipidemic subjects by centrifugation on a Ficoll-Hypaque gradient. The cells were incubated during 24 h in RPMI containing 100 units/ml of penicillin, 5 μg/ml of gentamicin, and supplemented with 10% of lipoprotein-deficient serum. This procedure induces the maximal expression of rLDL (2). After this period, the cells were harvested and the viability was evaluated by trypan blue exclusion. Viable cells (10⁶) were incubated with 10 μg of 14C-LDE-apo and increasing concentrations of LDL for 4 h at 37°C in 1 ml of RPMI. After incubation, the cells were washed three times with RPMI at 4°C and dissolved in 0.2 M NaOH for 2 h at 37°C. An aliquot was removed for protein determination (23) and the incorporated radioactivity was determined in the liquid scintillation counter.

Preparation of ⁹⁹mTc-LDE and ⁹⁹mTc-LDL. The procedure of labeling LDE with ⁹⁹mTc for imaging of its biodistribution in rabbits and patients had been developed and tested previously in rabbits. The technique was based on the procedure for obtaining labeled native LDL described by Lees et al. (24). LDE was labeled with ⁹⁹mTc using sodium dithionite as a reducing agent. The ⁹⁹mTc-LDL complex was purified by gel filtration and sterilized by passing through a 0.2-μm Millipore filter. ⁹⁹mTc-LDL (370 MBq) was injected i.v. into subjects within 30 min of radiolabeling. As tested by thin-layer chromatography, 90% of the radiation were found at the LDE phospholipids. LDL was also

---

4661
labeled with $^{99m}$Tc according to the technique of Lees et al. (24) for injection in rabbits.

**Clearance Studies in Rabbits.** Two groups of male New Zealand rabbits weighing 2-3 kg were given injections 111 MBq $^{99m}$Tc-LDE or $^{99m}$Tc-LDL via one ear vein and blood samples were collected from the other ear vein at preestablished intervals during 24 h. Plasma was separated by a 15-min centrifugation (3,000 × g) and radioactivity was determined in a gamma counter.

**Clearance Studies in Patients.** The participants were fasting at the commencement of the clearance studies, at approximately 9 a.m., but they were allowed two standard meals during the study, at approximately 12:30 p.m. and at approximately 7 p.m. They received injections of 37 kBq $^{14}$C-cholesteryl ester, roughly 5-6 mg of emulsion total lipids, at a volume of 500 μl, administered i.v. in a bolus. Plasma samples were collected during 24 h, in intervals of 5 min and 1, 2, 4, 6, 8, 12, and 24 h. Aliquots (1.5 ml) of blood plasma were extracted with chloroform:methanol (2:1, v/v) (25), and the solvent phase was transferred into counting vials and dried under a nitrogen stream. Radioactivity was measured in a scintillation solution (PPO:dimethyl-POPOP:Triton X-100:toluene, 5g:0.5g:333ml:667 ml) using a Beckman LS-100C spectrometer.

**Imaging Studies in Rabbits.** The planar images were acquired in anterior view 24 h postinjection of 111 MBq $^{99m}$Tc-LDE or $^{99m}$Tc-LDL. Gamma camera (Orbiter model; Siemens, Des Plaines, IL) images were obtained with a high resolution collimator and stored in 128 × 128 matrices in a dedicated computer system for subsequent analysis.

**Imaging Studies in Subjects.** The planar images were obtained in anterior and posterior views 6 h postinjection of 370 MBq $^{99m}$Tc-LDE. Digitized images of a view gamma camera (Siemens-Basicam, Des Plaines, IL) equipped with a general-purpose parallel-hole collimator were stored in 128 × 128 matrices in a dedicated computer system and analyzed later.

**Estimation of FCR.** FCR of the $^{14}$C-cholesteryl oleate of the LDE was calculated according to the method described by Matthews (26) as

$$
FCR = \frac{1}{y} (a_1 \cdot e^{-b_1 t} + a_2 \cdot e^{-b_2 t})^{-1}
$$

where $a_1$, $a_2$, $b_1$, and $b_2$ were estimated from biexponential curves obtained from the remaining radioactivity found in plasma after injection, fitted by least square procedure, as

$$
y = (a_1 \cdot e^{-b_1 t}) + (a_2 \cdot e^{-b_2 t})
$$

where $y$ represents the radioactivity plasma decay.

**Statistics.** Differences in LDE FCR and triglycerides between AML and ALL groups and before and after chemotherapy were assessed using the nonparametric Mann-Whitney test (27). Student's $t$ test was used to test for differences in values of total cholesterol and fractions, between the two groups and before and after hematological remission (27). Two-tailed $P$ values below 0.05 were considered significant. Pearson’s product moment correlation coefficient was used to assess the strength of association between LDE FCR and LDL-cholesterol and total cholesterol parameters.

**RESULTS**

**LDE Binding to Apolipoproteins.** Fig. 1 shows the sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels of the apolipoproteins that bound to LDE recovered after the incubation with HDL. Apo E was the predominant apolipoprotein that bound to LDE, albeit residues of other apolipoproteins can also be seen.

**Competition between LDE and LDL for Binding to Lymphocytes.** Fig. 2 shows that the uptake of $^{14}$C-cholesteryl oleate-labeled LDE by lymphocytes was progressively diminished as increasing LDL mass was added to the incubates. This indicates that LDE compete with LDL for the same receptor-mediated uptake mechanism.

**Comparison of LDE with LDL Plasma Kinetics in Rabbits.** Fig. 3 shows that $^{99m}$Tc-LDE and $^{99m}$Tc-LDL injected into the two groups of rabbits had similar plasma-decaying curves.

**Comparison of LDE with LDL Biodistribution Images Acquired in Rabbits.** The biodistribution images acquired from the two groups of four rabbits that received injections of $^{99m}$Tc-LDE or $^{99m}$Tc-LDL did not differ importantly. The images of two of those rabbits given injections of the two different preparations (Fig. 4) show that the uptake of radioactivity by the different organs is alike.

**Plasma Lipids and LDE Kinetics before Chemotherapy.** Table 1 shows the data for AML and ALL patients studied before chemotherapy. Plasma LDL-cholesterol was lower in the AML patients compared to the ALL patients ($P < 0.04$), but HDL-cholesterol and
triglyceride plasma concentration did not differ between the two groups. The cholesteryl ester FCR was approximately 3-fold greater in the AML patients than in the ALL patients 0.193 ± 0.210 (SD) and 0.666 ± 0.033 h⁻¹, respectively; P < 0.04 (Table 1; Fig. 5). It is noteworthy that the individual FCR values varied widely in AML, but not in ALL. A negative correlation was found between the cholesteryl ester FCR and the plasma concentration of total (r² = 0.33, P = 0.04) and LDL cholesterol (r² = 0.44, P = 0.02) in the AML but not in ALL group. In both, the cholesteryl esters FCR did not correlate with the plasma levels of HDL cholesterol and triglycerides or the blood leukocyte count.

**Plasma Lipids and LDE Kinetics in Hematological Remission.**

In hematological remission (Table 2), the plasma concentration of total and LDL cholesterol of AML patients rose 38 and 54%, respectively, compared to the pretreatment levels (P < 0.0025 for both parameters). The correlation between total and LDL cholesterol plasma concentration and FCR of the emulsion cholesteryl ester was no longer observed. Remarkably, the LDE removal from plasma decreased approximately 66% compared with the pretreatment results (from 0.193 ± 0.0210 to 0.065 ± 0.038 h⁻¹, P < 0.02; Fig. 6). In contrast, the LDE FCR did not change significantly in the ALL patients studied after remission, as well as their plasma levels of total and LDL cholesterol. HDL cholesterol levels remained unchanged in both groups after remission (Table 2).

**Biodistribution of LDE.** Fig. 7 shows the gamma camera images of the biodistribution of ⁹⁹mTc-LDE in one ALL and one AML nontreated patient, obtained 6 h postinjection. In the ALL subject, the liver was the predominant uptake site. LDE uptake by the liver was markedly lower in the AML patient (Fig. 7c) compared with the ALL patient (Fig. 7a). On the other hand, ⁹⁹mTc activity was pronouncedly higher in the AML patient over the areas corresponding to the pelvic bones (Fig. 7f), femur (Fig. 7g), and skull (Fig. 7h), the sites presumably containing bone marrow invaded by the malignant cells.

**DISCUSSION**

The major goal in cancer chemotherapy is to selectively kill the tumor cells without damaging normal tissues and organs. Therefore, the achievement of vehicles capable of delivering drugs to targeted cells has long been pursued. In the last decade, several authors suggested the possibility of using LDL to shuttle antitumor drugs to neoplastic cells, based on the increased expression of rLDL in certain types of cancer. Although it was successful in experimental trials, the difficulties in isolating LDL from plasma and handling the preparation are serious drawbacks for its use in routine treatment.

Protein-free emulsions can mimic the metabolism of plasma lipoproteins in vivo (28–32). Maranhão et al. (12) demonstrated in rats that LDE has a plasma kinetic behavior resembling that of native LDL and that the emulsion is probably taken up by the B,E receptors that sequestrate LDL into the cell. The structure and physical properties of protein-free microemulsions identical to LDE had been well defined previously (18, 33). LDE is devoid of apo B, but it acquires apo E from the circulating lipoproteins after injection into the blood. Because rLDL can also recognize apo E, LDE is thus taken up by the tissues via the LDL receptor-mediated endocytic pathway (12). In patients with familial hypercholesterolemia LDE plasma clearance was pronouncedly reduced compared to normal subjects (13). This is also expected for native LDL, because B,E receptors are defective in this disease (1).

The assumption that LDE is removed from the plasma by rLDL was strengthened in this study by the finding in rabbits that the ⁹⁹mTc-LDE...
plasma decay curve was similar to that of $^{99m}$Tc-LDL. Moreover, the biodistribution images in rabbits of both preparations showed a close resemblance, which also suggests that they are removed by the tissues by the same mechanism. Finally, the competition experiment indicates that LDE removal from the plasma of ALL patients equaled that of normolipidemic healthy subjects. $^c$ Again, LDE behaved like LDL, since the images acquired in the ALL patient resembled those obtained from two healthy subjects. $^d$ It is also noteworthy that the same trend occurred in AML. LDL-cholesterol concentration values, which were much more dispersed before than after the treatment. Among the nine AML patients who were studied twice, in only one (case 10) LDE FCR did not decrease posttreatment as expected, but otherwise it pronouncedly increased. Although this paradoxical behavior cannot be explained, it was again similar to LDL behavior, since that case was also the only one in whom LDL-cholesterol plasma concentration decreased instead of increasing in posttreatment.

LDE removal from the plasma of ALL patients equaled that of normolipidemic healthy subjects: LDE-FCR was 0.078 ± 0.033 h$^{-1}$, as observed in 19 healthy volunteers. $^3$ Moreover, the $^{99m}$Tc-LDE images acquired in the ALL patient resembled those obtained from two healthy subjects. $^3$ Again, LDE behaved like LDL, since the images obtained from the ALL patient and the two controls are similar to those obtained with $^{99m}$Tc-LDL (36), the liver being the main uptake organ. In the AML patient the lower hepatic uptake of $^{99m}$Tc-LDE associated with enhanced uptake over bone marrow areas was expected considering the overall results described in this study. Shift of the lower are the plasma levels of LDL, as shown in previous studies (34). Thus, the observed correlation suggests that LDE was removed from the circulation by the neoplastic cells with enhanced receptors that also remove the native lipoprotein (35). The lack of correlation in ALL and in AML after hematological remission further confirms this assumption.

We also demonstrated that when AML cells were removed from the blood and bone marrow by chemotherapy the LDE plasma clearance was pronouncedly reduced, approaching the values of the ALL patients. This confirms that the high clearance rates observed in AML patients before treatment were due to the presence of the neoplastic cells which avidly take up the emulsion particles. Since LDE FCR was reduced by 66% after the hematological remission, it can be estimated that approximately 66% of the emulsion injected into the nontreated patients was taken up by the AML cells, whereas only the remaining 34% was removed by the normal tissues. The lack of effect of chemotherapy on LDE clearance in the ALL patients agrees with these statements.

It is worthwhile to point out that there was a great interindividual variation in the LDE FCR measured in AML but not in ALL patients. This can be explained by the wide variation of rLDL expression in AML cells, which could range from a 3- to 100-fold increase (2, 14). It is remarkable that this variation became considerably smaller after remission, indicating that AML cells had been responsible for such variation in FCR. It is also noteworthy that the same trend occurred in AML LDL-cholesterol concentration values, which were much more dispersed before than after the treatment. Among the nine AML patients who were studied twice, in only one (case 10) LDE FCR did not decrease posttreatment as expected, but otherwise it pronouncedly increased. Although this paradoxical behavior cannot be explained, it was again similar to LDL behavior, since that case was also the only one in whom LDL-cholesterol plasma concentration decreased instead of increasing in posttreatment.
Fig 7. Biodistribution of LDE in one ALL (left) and one AML patient (right). Both had been recently diagnosed, and chemotherapy had not yet been started. In both bone marrow was hypercellular. The images were acquired 6 h after the injection of $^{99m}$Tc LDE: liver (a, e); pelvic bones (b, f); femur (c, g); skull (d, h).
the label from the liver to the bone marrow infiltrated by AML cells was also shown when $^{99m}$Tc-LDL was injected (37).

The clearcut results obtained in the present study fully confirmed our hypothesis that LDE can be a preparation perfectly suited for use as drug vehicle in routine cancer therapy. It is easy to prepare from materials produced by the chemical industry; hydrophobic drugs can be easily incorporated into it by cosonication with the lipid mixtures or incubation with the formed emulsion, and when stored at 4°C it can be preserved for a long period.

Moreover, the possibility of using LDE as a chemotherapeutic vehicle may not be limited to AML, since up-regulation of rLDL, the mechanism that allows targeting of cancer cells by both native LDL and LDE, was also observed in several other malignant neoplasias: glioma cells (38); endometrial adenocarcinoma (8); uterine cervical carcinoma (8); gallbladder cancer cells (39); breast cancer (40); myeloproliferative diseases (41); and metastatic prostate carcinoma (42).

ACKNOWLEDGMENTS

The authors are grateful to Dr. Odaly Tofololetto and Carmen G. Vinagre for help with the experiments; Maria C. M. Latriilha, Cristiane J. Furlanetto, and Jacques Malessy for expert technical assistance, and Julia T. Fukushima and Dr. Rita H. A. Cardoso for the statistical analysis.

REFERENCES

Plasma Kinetics and Biodistribution of a Lipid Emulsion Resembling Low-Density Lipoprotein in Patients with Acute Leukemia

Raul C. Maranhão, Bernardo Garicochea, Edson L. Silva, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/17/4660

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.